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(54) Title: USE OF HYALURONIDASE TO REDUCE VISCOELASTIC RELATED INCREASES IN INTRAOCULAR PRESSURE

(57) Abstract

Small doses, less than 15 IU and preferably less than 10 IU per treated eye, of hyaluronidase can safely and effectively be employed to reduce postoperative intraocular pressure caused by residual amounts of hyaluronan used during anterior segment surgical procedures. The hyaluronidase may be administered after surgery, or at 5 IU or less per treated eye concomitantly. Hyaluronidase treatment may be combined with treatments with other medications.

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USE OF HYALURONIDASE TO REDUCE VISCOELASTIC RELATED INCREASES IN INTRAOCULAR PRESSURE

FIELD OF THE INVENTION

This invention relates to the reduction of post-operative intraocular pressure spiking.

GOVERNMENT RIGHTS

The research leading to this invention was supported in part by United States government funds under Grant No. EY 00327 from the National Institutes of Health. Therefore, the U.S. Government has certain rights in this invention.

BACKGROUND OF THE INVENTION

The eye is divided into two major sections; the anterior segment which encompasses the crystalline lens and all structures in front of it; and the posterior segment which encompasses all structures behind the lens. The cornea, conjunctiva, iris, ciliary body, and the lens are all found in the anterior segment. In addition, the cavity defined by these structures within the anterior segment is sub-divided into anterior and posterior chambers. From front to back, the anterior chamber is the space between the iris, and the cornea, while the posterior chamber is the cavity between the iris and the lens. Peripherally, the anterior chamber is defined by the iridocorneal angle while the posterior chamber is circumscribed by the ciliary sulcus. This chamber system is filled with aqueous humor which is very similar to plasma (water, NaCl, K^+, etc.), is void of cells, and has little protein except in inflammatory or disease processes. The aqueous humor provides oxygen and nutrients to the lens, the corneal endothelium, and the trabecular meshwork, and carries metabolites away from many structures. It is secreted into the posterior chamber by the ciliary processes

and then flows into the anterior chamber through the pupillary opening. The aqueous humor leaves the eye through the trabecular meshwork in the iridocorneal angle and Schlemm's canal. Crisscrossed collagen beams covered by endothelial cells form the meshwork. The aqueous humor drains through the meshwork and into the Schlemm's canal which is a small vessel in the sclera. The tissue between the inner wall of the Schlemm's canal and the corneoscleral meshwork is responsible for most of the resistance to outflow of aqueous humor in the outflow pathway. Abnormal resistance to outflow of aqueous humor leads to an increase in intraocular pressure, which is believed to be the cause of open angle glaucoma.

The posterior segment of the eye consists of the retina with the optic nerve, the vitreous, the choroid, and the posterior portion of the sclera. The vitreous fills the cavity between the lens and the retina. The vitreous humor is a connective tissue composed of a small amount of glycosaminoglycans, collagen and a large amount of water. In the human eye, the vitreous consists of the cortical layer, the intermediate zone, and the central zone. The closest area to the retina is the cortical vitreous, which consists of collagen and hyaluronic acid and has a gel-like consistency. The cells of the vitreous, called hyalocytes, are dispersed in the outer parts of the cortical vitreous. The water content of the vitreous is as high as 98 to 99.7%. As the vitreous is an avascular structure, there is no flow through the vitreous, only diffusion. The blood vessels in the retina, the pigment epithelium, and the choroid are also barriers to drug penetration into the retina and the vitreous.

Hyaluronan, also known as hyaluronic acid or sodium hyaluronate, is a naturally occurring viscoelastic compound found in many tissues of the body, including the synovial fluid and vitreous. This material, in different commercial formulations, is often used in anterior and posterior segment

surgical procedures to prevent damage to tissue surfaces and to allow for more space during surgical manipulation; as a transparent substance, hyaluronan does not interfere with intraoperative visibility. Viscoelastics of several kinds have been used, e.g., in cataract surgery to maintain the shape of the anterior chamber, during lens implantation and phacoemulsification, and to protect the corneal endothelium from mechanical trauma and contact with nearby tissues and surgical instruments. This use has resulted in significant decreases in complication rates and has dramatically increased the safety of surgical procedures.

Despite their advantages, the use of viscoelastics has been correlated with significant increases in intraocular pressure postoperatively. The viscoelastic can remain in the anterior chamber, in sufficient amount to cause postoperative problems, for up to 6 days and, as a result, inhibit the elimination of anterior-chamber fluid, inflammatory products and inflammatory cells. Dangerous post-operative pressure spikes can occur before elimination of viscoelastic is completed. These intraocular pressure spikes can be caused by any of the viscoelastics and are apparent as early as 3 hours after the surgery. The pressure spikes can last many days, with pressures reaching as high as 60 mm Hg. Such spikes cause severe headaches and nausea and may potentially complicate blood flow, causing vision-threatening ocular ischemia and tissue damage. While strategies have been devised to minimize intraocular pressure elevations, including thorough washout of the anterior chamber at the end of surgery, routine use of anti-glaucoma medicines post-operatively, and postoperative paracentesis, none is ideal. A complete evacuation of viscoelastics is often not practical given the current technique of small incision cataract surgery. Anti-glaucoma medicines can have significant side-effects of their own, and frequently are not effective in reducing viscoelastic related pressure rises. Finally, postoperative paracentesis requires that a second procedure

be performed soon after primary surgery. Satisfactory methods in reducing viscoelastic related rises in intraocular pressure are lacking.

Hyaluronidase, e.g., Wydase®, is currently used for subcutaneous/retrobulbar injections of local anesthesia to promote the diffusion of anesthetics. Hyaluronidase is a highly specific naturally occurring enzyme which cleaves hyaluronan into disaccharide components, thus reducing both its viscosity and molecular weight. In 1986, Calder and Smith (British J. Ophthalmol. 70: 418-420, 1986) injected 750 IU of hyaluronidase into the anterior chamber with sodium hyaluronate during intracapsular cataract extraction in human subjects. Pigment dispersion was observed in the anterior chamber in one of the 10 eyes, while 2 of the 10 patients developed cystoid macular edema. This adverse event rate suggests that hyaluronidase could not be used for this indication. Hein et al. (Ophthalmic Surgery 17:731-734, 1986) injected 5, 10, 25, 50 and 150 IU of *Streptomyces* derived hyaluronidase into the anterior chamber of live rabbit eyes. No abnormalities were reported on gross and histologic examinations after 48 hrs. Subsequently, rabbits were treated in the anterior chamber of one eye with a 10% solution of hyaluronan of unknown molecular weight and 20 IU of *Streptromyces* hyaluronidase, and the treated eye was compared to a control. Tonography, to determine outflow facility rather than intraocular pressure, was performed at 12, 24 and 48 hrs. No significant change in outflow facility was noted at any time interval.

In 1990, Gottlieb et al. (Invest. Ophthalmol. Vis. Sci. 31: 2345-2352, 1990) used different concentrations of bovine testicular hyaluronidase (1, 15, 30, 50, and 150 IU) in the posterior segment for enzymatic vitreolysis. The vitreous humor is a connective tissue between the retina and lens composed 98 to 99.7% of water. The small remaining percentage is made up of collagen and hyaluronic acid. In studying the safety of injecting hyaluronidase

intravitreally, they found clinical and histologic abnormalities at all concentrations above 15 IU.

SUMMARY OF THE INVENTION

We have shown, surprisingly, that in small doses, i.e., less than 15 (and more preferably less than 10) IU per treated eye, hyaluronidase can safely and effectively be employed to blunt the postoperative intraocular pressure spikes normally caused by residual amounts of the viscoelastic hyaluronan used during anterior segment surgical procedures. Furthermore, in very low doses, e.g., less than 5 IU per treated eye, hyaluronidase can be added at the same time as the required hyaluronan, for additional beneficial effects. If the viscoelastic is in sufficient excess over the degredative enzyme, its protective effect will be maintained during the critical surgical time period. Yet viscoelastic degradation will begin sufficiently early to minimize intraocular pressure elevations.

In addition, hyaluronidase administration, in small doses either during or subsequent to surgery, can be combined with that of other medication. For example, the concomitant use of anti-glaucoma medication, e.g., acetazolamide (Diamox), during intraocular lens replacement or other procedure, would be appropriate, e.g., if it were known that the patient had glaucoma, if undiagnosed glaucoma were a possibility, if there were damage to the optic nerve or if the patient also had cataracts. Alternatively, the appropriate dosage of hyaluronidase can be injected at the end of the surgical procedure, simultaneously with the injection of, e.g., acetylcholine (Miochol) or other agent used to return the pupil to normal size, so that no modification in surgical procedure is necessary.

As another alternative, hyaluronidase could be added to the infusion fluid (e.g., Balanced Salt Solution) routinely used during surgery to maintain a formed anterior chamber.

BRIEF DESCRIPTION OF THE DRAWINGS

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims, taken in conjunction with the accompanying drawings, in which:

Fig. 1 shows the average changes of intraocular pressure in eyes treated with various viscoelastic substances (Control Eyes);

Fig. 2 shows the effect of hyaluronidase alone on intraocular pressure;

Figs. 3a and 3b show the effects of the viscoelastic Healon® on intraocular pressure with and without the addition of 5 IU and 10 IU hyaluronidase, respectively; and

Figs. 4-6 show the effects of the viscoelastics Healon GV®, Viscoat® and Ocucoat®, respectively, on intraocular pressure, with and without the addition of 10 IU hyaluronidase.

DETAILED DESCRIPTION OF THE INVENTION

The ability of hyaluronidase to prevent increases in intraocular pressure provides for a method of preventing viscoelastic related post operative pressure spikes. This enzyme can be instilled in the anterior chamber of the eye of a patient at some point after hyaluronan containing viscoelastic has been used during anterior segment surgery. Use of hyaluronidase to degrade the hyaluronan obviates the need to evacuate the viscoelastic completely following surgery.

Essentially, in practicing the method of the invention, a practitioner would begin by calculating the risk of a patient in getting postoperative increases in intraocular pressure following surgery. If a patient has a high risk of ocular damage from such IOP increases (e.g., a glaucoma patient or a patient with retinal vascular disorders, or a very old patient), then the practitioner may administer the hyaluronidase during the surgery. The surgeon would decide

either to dilute 5-10 units of hyaluronidase into the infusion bottle to be used throughout the procedure or, alternatively, to give 5-10 units of hyaluronidase as a separate injection (or perhaps combined with Miochol) towards the end of the procedure. One further option may be adding 1-5 units of hyaluronidase to hyaluronan (which may be mixed at the time of hyaluronan's administration) and then injecting this hyaluronan/hyaluronidase combination. If the surgeon feels that increases in intraocular pressure are not likely or are inconsequential, he/she may omit such injections and use hyaluronidase the next day, should IOP increase post-operatively.

The following examples are presented to illustrate the advantages of the present invention and to assist one of ordinary skill in making and using the same. These examples are not intended in any way otherwise to limit the scope of the disclosure.

EXAMPLE I

Three commercially available hyaluronan containing products, Healon®, Healon GV® and Viscoat®, and one hydroxypropyl methylcellulose product, Ocucoat®, were tested with and without hyaluronidase in a rabbit model system to examine the efficacy of treatment with low concentrations of hyaluronidase in reducing viscoelastic-induced intraocular pressure spiking without unwanted side effects. The properties and sources of the individual compounds used are indicated in the following table.

Table I

Group	Product	Brand Name	Manufacturer	Viscosity (cpm)
I	Sodium Hyaluronate 1%	Healon®	Pharmacia & Upjohn, Kalamazoo,MI	200,000
II	Sodium Hyaluronate 1.4%	Healon GV®	Pharmacia & Upjohn, Kalamazoo,MI	2,000,000
III	Sodium Hyaluronate 3% and Chondroitin Sulfate 4%	Viscoat®	Alcon Laboratories Fort Worth, TX	41,000
IV	Hydroxypropyl methylcellulose 2%	Ocucoat®	Storz Ophthalmics, St. Louis, MO	5,400
V	Hyaluronidase	Wydase®	Wyeth, Philadelphia, PA	

Twenty-five white New Zealand rabbits (weight 2.0-3.4 Kg) were included in this study. Throughout the study, the procedures in the ARVO statement on the Use of Animals in Ophthalmic and Vision Research were strictly adhered to. At the onset of the investigation, each eye of all rabbits was examined by slit lamp biomicroscopy to exclude any preexisting abnormality.

Rabbits were anesthetized with intramuscular injection of a combination of ketamine (50 mg/Kg) and 0.5 ml of chlorpromazine HCl (100 mg/ml). Proparacaine HCl (0.5%) eye drops were instilled into the conjunctival cul de sac 1 minute prior to injection. Preoperative intraocular pressures were measured using a tonopen in each eye. The lids were held open using a wire speculum and after the eyes

were immobilized, a syringe fitted with a 30G needle was inserted at the limbus into the anterior chamber and 0.22 ml of aqueous fluid was removed. Next, using a separate 30G needle, 0.20 ml of viscoelastic substance was injected into the anterior chamber of the right eye. Using the same needle, 0.02 ml of hyaluronidase (either 5 or 10 IU, as indicated below) was injected in the anterior chamber of the same eye. The anterior chamber of the left eye, serving as control, was injected with equivalent volumetric dose (0.22 ml) of the same viscoelastic substance. Rabbits were divided into 5 groups of 5 each. The right eye of each group was injected with a separate viscoelastic substance, as indicated in Table I. In group V, the right eye was injected with 10 IU of hyaluronidase, and the left eye, serving as control, was injected with 0.22 ml of Balanced Salt Solution (Alcon Surgical).

Immediately after injections, rabbits were examined by biomicroscopy to evaluate possible ocular trauma caused by the injections. Intraocular pressures were measured using tonopen tonometry immediately after injection. Subsequent tonometry was done by using topical anesthesia using proparacaine 0.5%. Tonometry was repeated at 1, 2, 4, 6, 8, 12, 24, and 48 hours after injection. Ocular examination was performed using slit lamp biomicroscopy and indirect ophthalmoscopy to evaluate the anterior chamber, lens, vitreous and retina. The data were analyzed statistically using the one and two tailed paired student t-test. At the completion of the study period, all rabbits were killed with a lethal dose of pentobarbital (100 mg/kg given in the marginal ear vein).

Referring to Fig. 1, the average changes of intraocular pressure in control eyes treated with all four indicated viscoelastic substances can be seen. Intraocular pressure increased within 1 hour after anterior chamber injection of the respective viscoelastic and reached a peak at approximately 4-6 hours post injection. Intraocular pressure

gradually decrease to approximate pre-injection levels at about 24 hours post-injection. An increase in intraocular pressure occurred with all of the viscoelastics evaluated. Healon GV® appeared to have the most profound increase in intraocular pressure ($p<0.05$ at 2, 4, and 6 hours post injections) compared to other viscoelastics.

Fig. 2 shows the intraocular pressures obtained after 10 IU of hyaluronidase were injected in the anterior chamber of the right eye. Compared to control (left eye), hyaluronidase alone appears to have brought about a modest decrease in intraocular pressure in the experimental eye. These results were statistically significant only at 4 and 48 hours post injections ($p<0.05$).

Figs. 3a-6 summarize the results of intraocular pressure changes when Healon®, Healon GV®, Viscoat®, and Ocucoat® were used in the presence (treated right eye) and absence (control left eye) of hyaluronidase. Differences in intraocular pressure between the treated and control eyes were assessed by the unequal paired student t-test. Referring to Figs. 3a and 3b, 5 IU of hyaluronidase reduced the Healon® produced intraocular pressure maximum almost by half, while treatment with 10 IU of hyaluronidase eliminated the IOP spike seen with Healon® alone. As shown in Figs. 4 and 5, the results were similar with Healon GV® and Visicoat®; 10 units of hyaluronidase substantially eliminated the viscoelastic produced rise in intraocular pressure. However, as shown in Fig. 6, when the hydroxypropyl methylcelllulose product Ocucoat® was tested with and without hyaluronidase, intraocular pressure increases were essentially identical. Thus, it is apparent that hyaluronan-containing viscoelastic related rises in intraocular pressures can be prevented with concomitant use of low doses of hyaluronidase without apparent ill effects.

While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. A method for reducing post-operative intraocular pressure spiking following anterior segment surgical procedures, said method comprising:

providing a patient having an anterior segment surgical procedure performed on an eye;

administering a composition comprising hyaluronan to the eye of said patient during said surgical procedure; and

administering a composition comprising hyaluronidase to the eye of said patient during said surgical procedure, wherein the total dose of hyaluronidase administered is less than 15 IU per treated eye.

2. The method of claim 1 wherein the total dose of hyaluronidase administered is less than 10 IU per treated eye.

3. The method of claim 1 wherein the total dose of hyaluronidase administered is less than 5 IU per treated eye.

4. The method of claim 3 wherein said composition comprising hyaluronidase is administered to the treated eye of said patient symultaneously with said composition comprising hyaluronan.

5. The method of claim 1 wherein said composition comprising hyaluronidase is administered to the treated eye of said patient at the end of said surgical procedure.

6. The method of claim 1 wherein said composition comprising hyaluronidase is administered to the treated eye of said patient in a combination with other medication.

7. The method of claim 6 wherein said other medication comprises anti-glaucoma medication.

8. The method of claim 6 wherein said other medication comprises acetylcholine and said composition comprising hyaluronidase is administered at the end of said surgical procedure.

9. The method of claim 1 wherein said composition comprising hyaluronidase is administered to the treated eye of said patient via injection.

10. The method of claim 1 wherein said composition comprising hyaluronidase is administered to the treated eye of said patient in infusion fluid.

11. A method for reducing post-operative intraocular pressure spiking following anterior segment surgical procedures, said method comprising:

providing a patient having an anterior segment surgical procedure performed on an eye;

administering a composition comprising hyaluronan to the eye of said patient during said surgical procedure; and

administering a composition comprising hyaluronidase to the eye of said patient following said surgical procedure, wherein the total dose of hyaluronidase administered is less than 15 IU per treated eye.

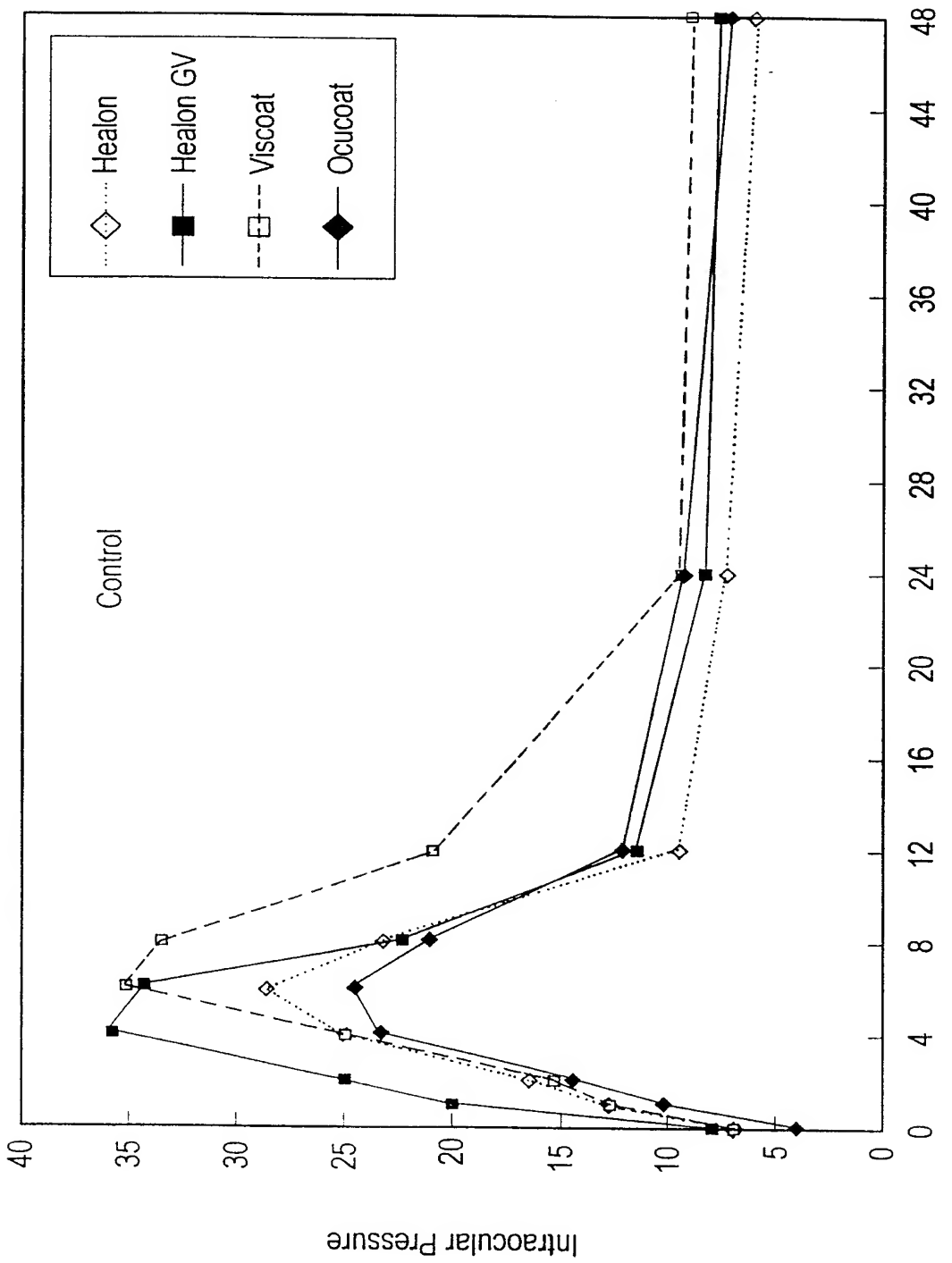
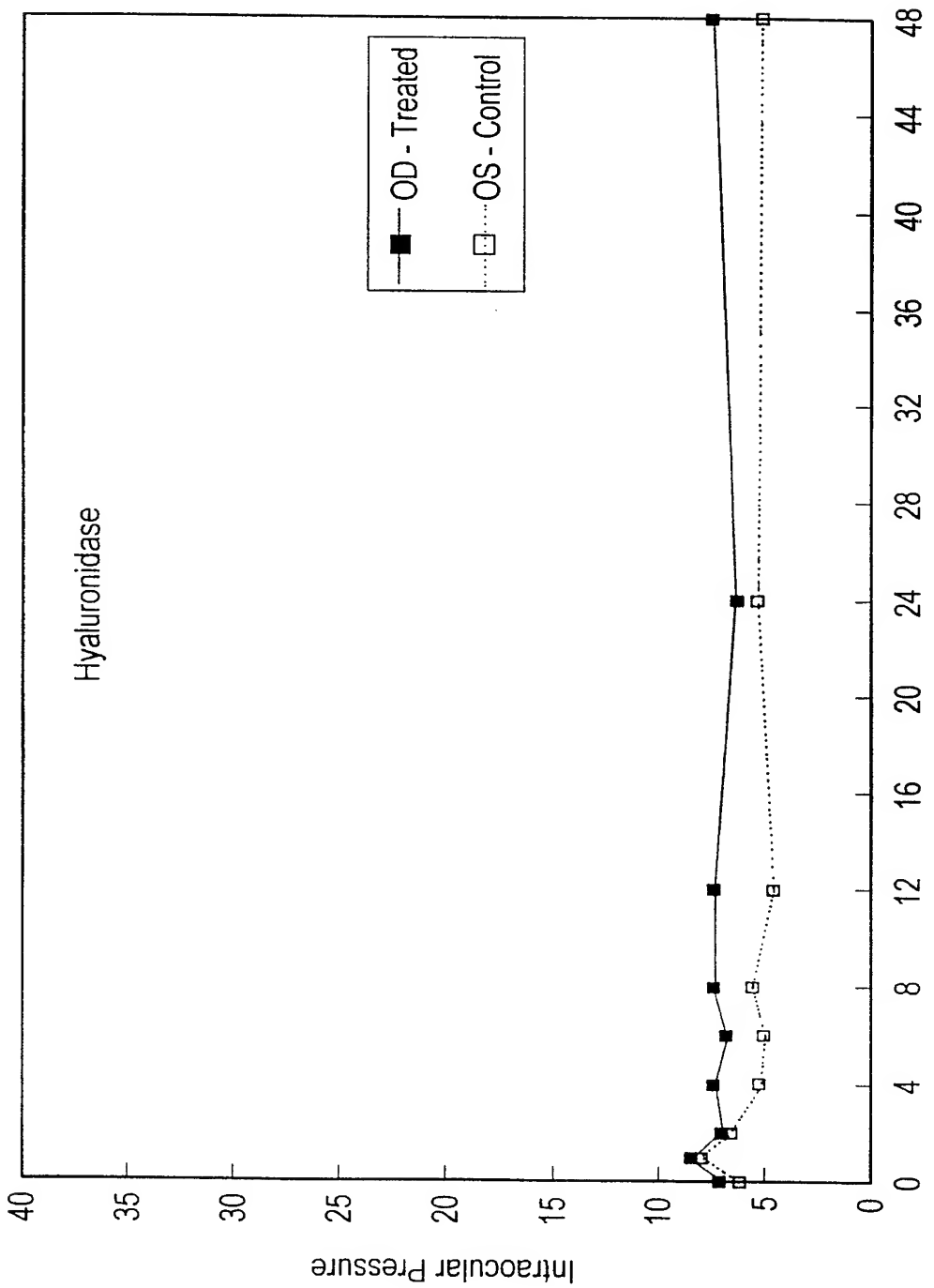

Healon
Healon GV
Viscoat
Ocucoat
Control
Intraocular Pressure
TIME (hours)
0
4
8
12
16
20
24
28
32
36
40
44
48
0
5
10
15
20
25
30
35
40

FIG. 1


Hyaluronidase
OD - Treated
OS - Control
Intraocular Pressure
Time (hours)
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8
12
16
20
24
28
32
36
40
44
48
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5
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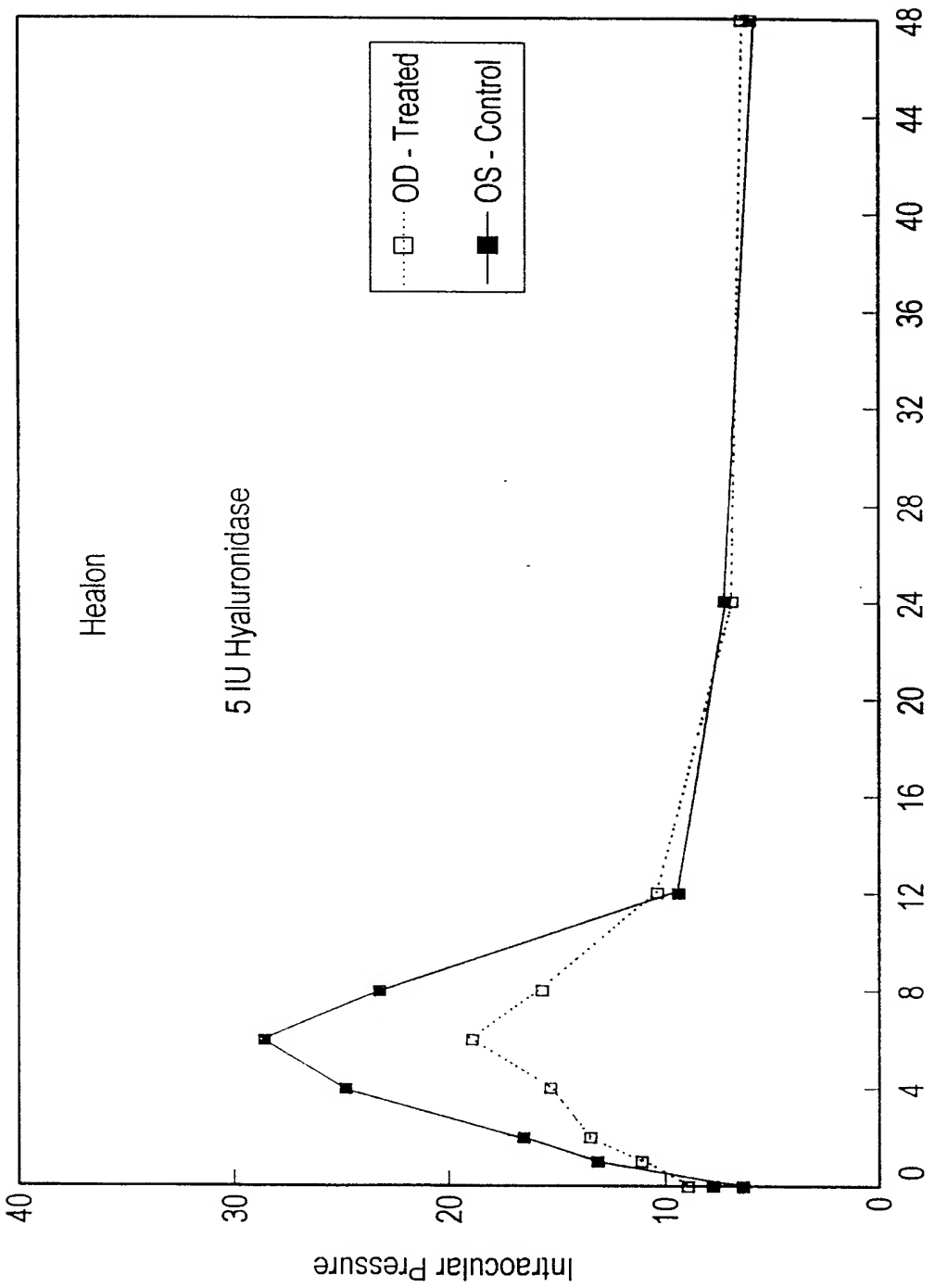
35
40

FIG. 2


Healon
5 IU Hyaluronidase
OD - Treated
OS - Control
Intraocular Pressure
Time (hours)
0
10
20
30
40
0
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36
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44
48

FIG. 3a

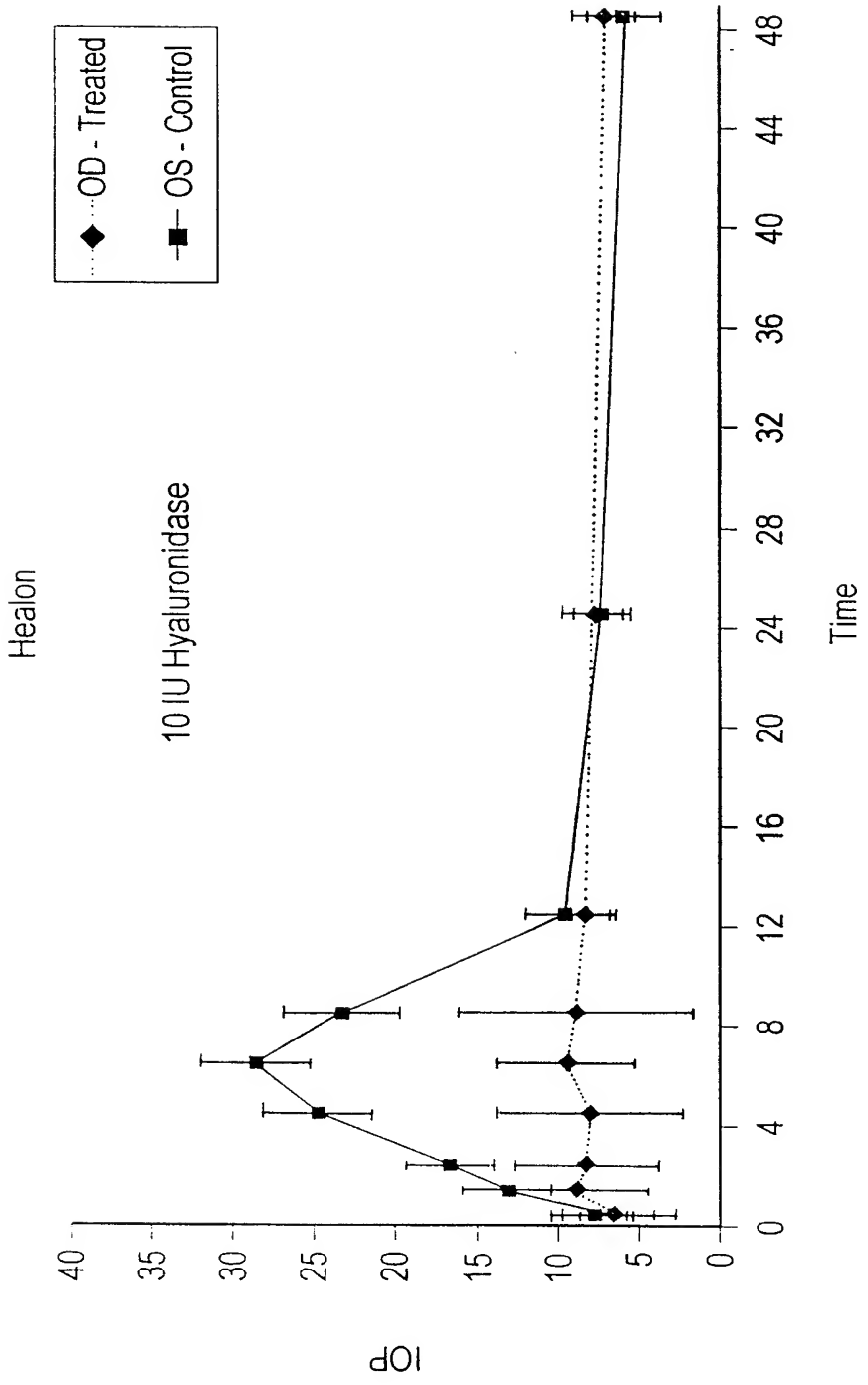
OD - Treated
OS - Control
Healon
10 IU Hyaluronidase
IOP
Time
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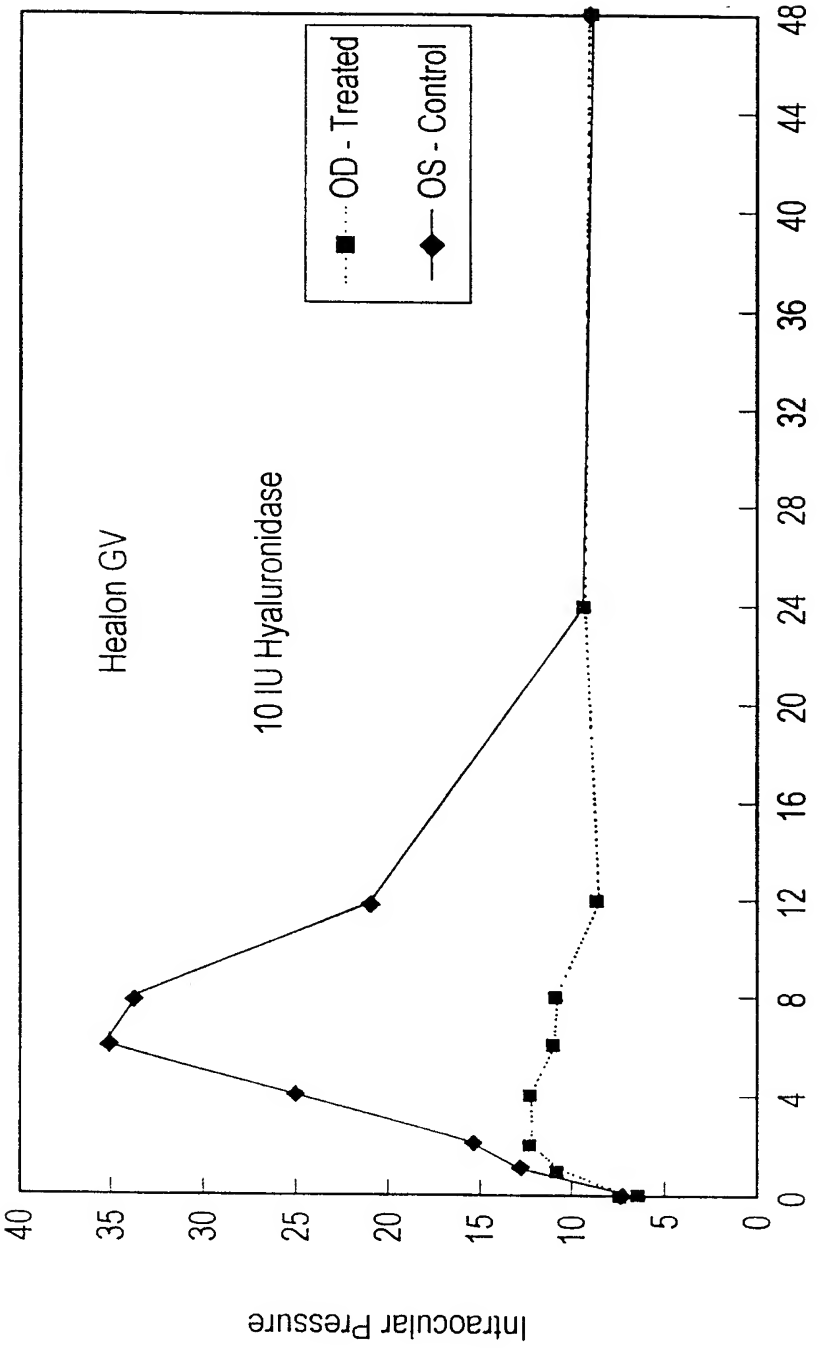
FIG. 3b


Healon GV
10 IU Hyaluronidase
OD - Treated
OS - Control
Intraocular Pressure
Time (hours)
0
5
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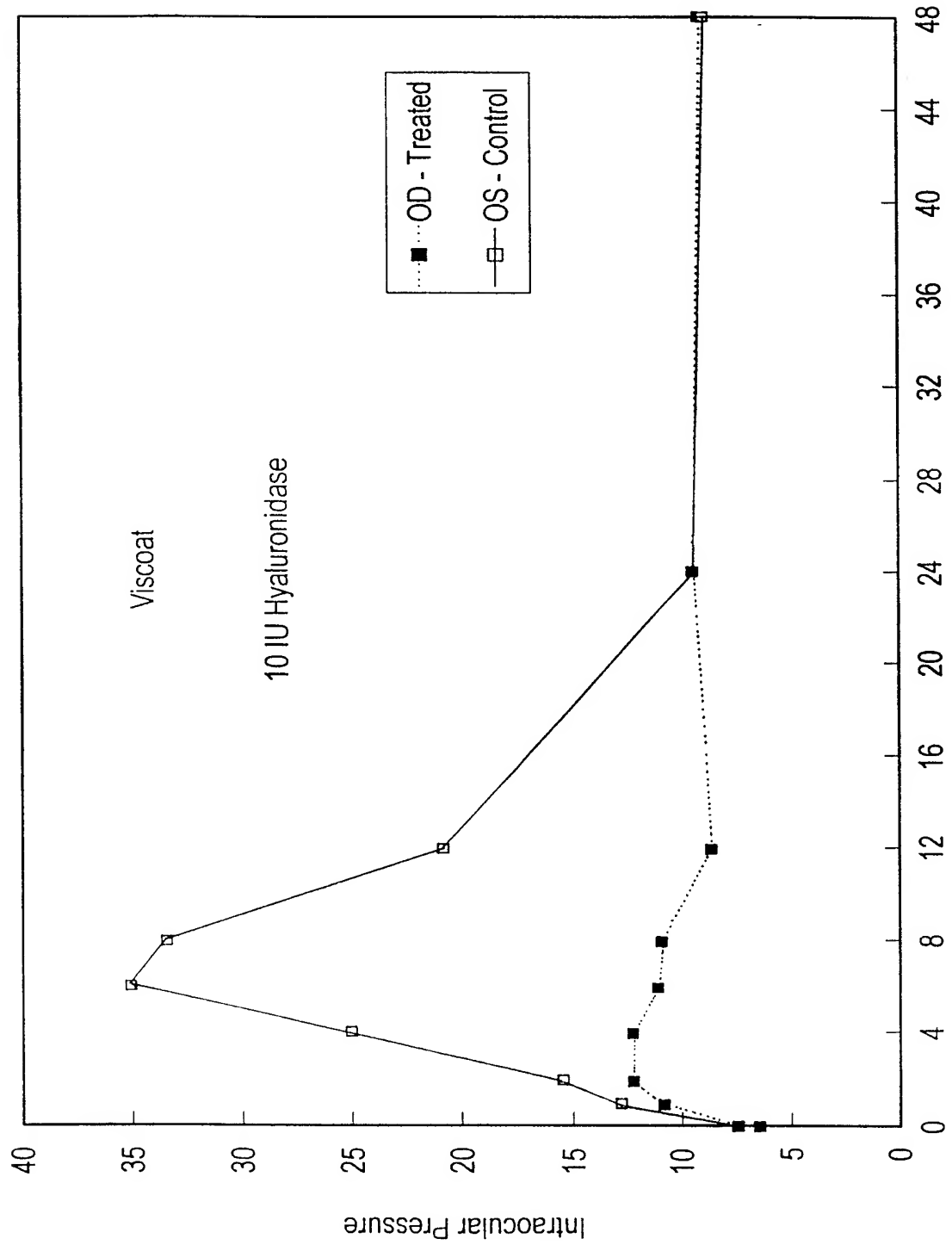
48

FIG. 4


Viscoat
10 IU Hyaluronidase
OD - Treated
OS - Control
Intraocular Pressure
Time (hours)
0
5
10
15
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0
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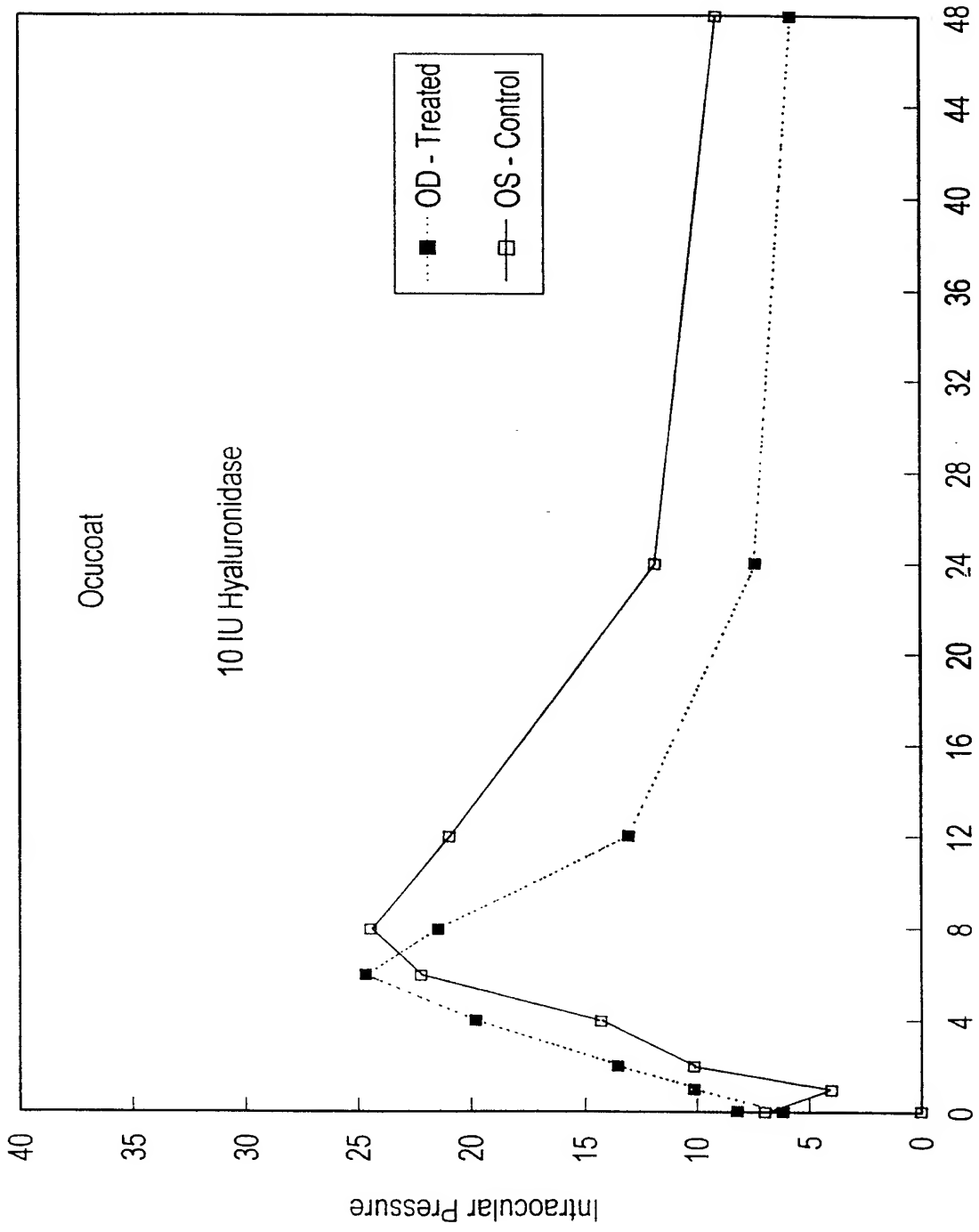
FIG. 5


Ocucoat
10 IU Hyaluronidase
OD - Treated
OS - Control
Intraocular Pressure
Time (hours)
0
5
10
15
20
25
30
35
40
0
4
8
12
16
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24
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32
36
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44
48

FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03125

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/46; C12N 9/26; A01N 43/04
US CL :424/94.62; 435/201; 514/54

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/94.62; 435/201; 514/54

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EQUI et al. Hyaluronan Polymer Size Modulates Intraocular Pressure. Journal of Ocular Pharmacology and Therapeutics. August 1997, Vol. 13, No. 4, pages 289-295, see entire document.	1-11
X	CALDER et al. Hyaluronidase and Sodium Hyaluronate in Cataract Surgery. British Journal of Opthalmology. June 1986, Vol. 70, No. 6, pages 418-420, see entire document.	1-11
X	RANKOVA et al. Application of Hyaluronidase After Unsuccessful Trabeculectomy. Documenta Ophthalmologica. 1992, Vol. 80, No. 4, pages 381-383, see entire document.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 31 MARCH 1999	Date of mailing of the international search report 22 APR 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer JON P. WEBER, PH.D. Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03125

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LANG et al. Shear Flow Characteristics of Sodium Hyaluronate. Arch. Opthalmol. July 1984, Vol. 102, No. 7, pages 1079-1082, see entire document.	1-11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/03125

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

REG, MEDLINE, BIOSIS, EMBASE, WPIDS, CAPLUS

search terms: hyaluronidase (and chemical names from REG), eye, ocular, intraocular, surgery, surgical, operative, operation